Serial No.: 10/042,421 Filed: October 18, 2001

Page : 2 of 18

Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

- 1. (currently amended) A preparation of a substantially purified glycosylated CD44 polypeptide, said glycosylated CD44 polypeptide comprising an amino acid sequence encoded by a nucleotide sequence comprising exons 1-5, 16, 18, and 20 of a human CD44 gene at least 95% identical to SEQ ID NO: 1 or to a sequence of a CD44 isoform arising from alternative splicing, wherein the CD44 polypeptide is a human CD44H isoform, a human CD44R1 isoform, or a human CD44R2 isoform, [[and]] wherein said glycosylated CD44 polypeptide binds to an antibody having the binding specificity of monoclonal antibody HECA-452, and wherein the preparation comprises less than 30% of a polypeptide other than the glycosylated CD44 polypeptide.
- 2. (currently amended) The preparation 1 of claim 1, wherein binding of said glycosylated polypeptide to said antibody decreases following contacting of said glycosylated polypeptide with N-glycosidase-F under conditions sufficient to remove carbohydrate moieties from said glycosylated polypeptide.
- 3. (previously presented) The preparation of claim 1, wherein binding of said glycosylated polypeptide to said antibody decreases following contacting of said glycosylated polypeptide with sialidase under conditions sufficient to remove sialic acid moieties from said glycosylated polypeptide.
- 4. (previously presented) The preparation of claim 1, wherein binding of said glycosylated polypeptide to said antibody decreases following contacting of said glycosylated polypeptide

Serial No.: 10/042,421 Filed: October 18, 2001

D--- 2 . C10

Page : 3 of 18

with fucosidase under conditions sufficient to remove fucose moieties from said glycosylated polypeptide.

5. (previously presented) The preparation of claim 1, wherein said glycosylated polypeptide is an E-selectin ligand, wherein the E-selectin ligand activity is mediated by a N-linked carbohydrate moiety on said glycosylated polypeptide.

- 6. (currently amended) The preparation of claim 1, wherein said glycosylated polypeptide is an L-selectin ligand, wherein the L-selectin ligand activity is mediated by N-linked carbohydrate moitiey moiety on said glycosylated polypeptide.
- 7. (previously presented) A preparation of a substantially purified glycosylated polypeptide comprising the amino acid sequence of SEQ ID NO: 1, and wherein said glycosylated polypeptide binds to an antibody having the binding specificity of monoclonal antibody HECA-452.
- 8. (withdrawn) A method for identifying a stem cell, the method comprising: (a) contacting a test cell population with one or more agents that specifically bind to the glycosylated polypeptide of claim 1 under conditions sufficient to form a complex between said agent and stem cell, if present, in said population; and (b) detecting said complex, thereby identifying said stem cell.
- 9. (withdrawn) The method of claim 8, wherein said one or more agents is an anti-CD44 antibody.
- 10. (withdrawn) The method of claim 8, wherein said one or more agents is an antibody with the binding specificity of monoclonal antibody HECA-452.
- 11. (withdrawn) The method of claim 8, wherein said at least one or more agents is an antibody

Serial No.: 10/042,421 Filed: October 18, 2001

Page : 4 of 18

with the binding specificity of monoclonal antibody HECA-452.

12. (withdrawn) A method for identifying a stem cell, the method comprising: (a) providing a E-selectin polypeptide immobilized on a solid phase; (b) contacting the solid phase with a fluid sample containing a suspension of test cells wherein the relative movement between the solid phase and the fluid sample is such that shear stress is achieved at the surface of the solid phase; and (c) observing the test cells that adhere to the solid phase thereby identifying said stem cell.

- 13. (withdrawn) A method for identifying a stem cell, the method comprising: (a) providing a L-selectin polypeptide immobilized on a solid phase; (b) contacting the solid phase with a fluid sample containing a suspension of test cells wherein the relative movement between the solid phase and the fluid sample is such that shear stress is achieved at the surface of the solid phase; and (c) observing the test cells that adhere to the solid phase thereby identifying said stem cell.
- 14. (withdrawn) The method of claim 12 or 13, wherein said shear stress is greater than 0.6 dynes/cm².
- 15. (withdrawn) The method of claim 12, wherein said shear stress is at least 2.8 dynes/cm².
- 16. (withdrawn) The method of claim 13, wherein said shear stress is at least 10 dynes/cm².
- 17. (withdrawn) The method of claim 8, 12 or 13, wherein said test cell is blood.
- 18. (withdrawn) The method of claim 8, 12 or 13, wherein said test cell is bone marrow.
- 19. (withdrawn) A method of isolating a stem cell from a population of cells, the method comprising: (a) contacting a cell population with one or more agents that specifically bind to the glycosylated polypeptide of claim 1 under conditions sufficient to form a complex between said

Serial No.: 10/042,421 Filed: October 18, 2001

Page : 5 of 18

one or more agents and a stem cell, if present, in said population of cells; (b) detecting said complex; (c) removing said complex from said cell population, thereby isolating said stem cell from said cell population

- 20. (withdrawn) The method of claim 19, further comprising separating said stem cell from said one or more agents, thereby disrupting said complex.
- 21. (withdrawn) A method of isolating a stem cell from a population of cells, the method comprising: (a) providing a E-selectin polypeptide immobilized on a solid phase; (b) contacting the solid phase with a fluid sample containing a suspension of cells wherein the relative movement between the solid phase and the fluid sample is such that shear stress is achieved at the surface of the solid phase; and (c) recovering the cells that adhere to the solid phase thereby isolating said stem cell.
- 22. (withdrawn) A method of isolating a stem cell from a population of cells, the method comprising: (a) providing a L-selectin polypeptide immobilized on a solid phase; (b) contacting the solid phase with a fluid sample containing a suspension of cells wherein the relative movement between the solid phase and the fluid sample is such that shear stress is achieved at the surface of the solid phase; and (c) recovering the cells that adhere to the solid phase thereby isolating said stem cell.
- 23. (withdrawn) The method of claim 21 or 22, wherein said shear stress is greater than 0.6 dynes/cm².
- 24. (withdrawn) The method of claim 21, wherein said shear stress is at least 2.8 dynes/cm².
- 25. (withdrawn) The method of claim 22, wherein said shear stress is at least 10.0 dynes/cm².

Attorney's Docket No.: 10286-014001 / BWH-729

Applicant: Robert Sackstein Serial No.: 10/042,421 Filed: October 18, 2001

Page : 6 of 18

26. (withdrawn) A method of treating a hematopoietic disorder in a mammal, the method comprising administering to said mammal a composition comprising the cells isolated according to the method of claim 19, 21 or 22.

- 27. (withdrawn) A method of treating cancer in a mammal the method comprising administering to said mammal a composition comprising the cells isolated according to the method of claim 19, 21 or 22.
- 28. (withdrawn) A method of increasing the affinity of a cell for E-selectin and/or L-selectin, the method comprising (a) providing said cell; and (b) contacting said cell with one or more agents that increases cell-surface expression or activity the glycosylated polypeptide of claim 1 on said cell, thereby increasing affinity of said cell for E-selectin and/or L-selectin.
- 29. (withdrawn) The method of claim 28, wherein said cell is a stem cell.
- 30. (withdrawn) The method of claim 28, wherein said one or more agents is a nucleic acid that encodes a CD44 polypeptide.
- 31. (withdrawn) The method of claim 28, wherein said one or more agents is a nucleic acid that encodes a glycosyltransferase or a glycosidase polypeptide.
- 32. (withdrawn) The method of claim 28, wherein at least one or more of said agents is a nucleic acid that encodes a glycosyltransferase.
- 33. (withdrawn) A method of increasing the engraftment potential of a stem cell, the method comprising: (a) providing said stem cell; and (b) contacting said stem cell with one or more agents that increases cell-surface expression or activity of the glycosylated polypeptide of claim 1 on said cell, thereby increasing the engraftment potential of stem cell.

Applicant: Robert Sackstein Serial No.: 10/042,421 Filed: October 18, 2001

Page : 7 of 18

34. (withdrawn) The method of claim 33, wherein said one or more agents is a nucleic acid that encodes a CD44 polypeptide.

- 35. (withdrawn) The method of claim 33, wherein said one or more agents is a nucleic acid that encodes a glycosyltransferase or a glycosidase polypeptide.
- 36. (withdrawn) The method of claim 33, wherein at least one or more of said agents is a nucleic acid that encodes a glycosyltransferase.
- 37. (withdrawn) A method of increasing the engraftment potential of a cell population, the method comprising: (a) providing a L-selectin polypeptide immobilized on a solid phase; (b) contacting the solid phase with a fluid sample containing said cell population, wherein the relative movement between the solid phase and the fluid sample is such that shear stress is achieved at the surface of the solid phase; and (c) recovering the cells that adhere to the solid phase thereby increasing the engraftment potential of a cell population.
- 38. (withdrawn) A method of increasing the engraftment potential of a cell population, the method comprising: (a) providing a E-selectin polypeptide immobilized on a solid phase; (b) contacting the solid phase with a fluid sample containing said cell population, wherein the relative movement between the solid phase and the fluid sample is such that shear stress is achieved at the surface of the solid phase; and (c) recovering the cells that adhere to the solid phase thereby increasing the engraftment potential of a cell population.
- 39. (withdrawn) A method of increasing levels of engrafted stem cells in a subject, the method comprising administering to said subject an agent that increases cell-surface or expression of the glycosylated polypeptide of claim 1 on one or more stem cells in said subject.

Serial No.: 10/042,421 Filed: October 18, 2001

Page : 8 of 18

40. (withdrawn) A method of increasing levels of engrafted stem cells in a subject, the method comprising administering to said subject a composition comprising the cells isolated according to the method of claim 33, 37 or 38.

- 41. (withdrawn) The method of claim 37 or 38, wherein said subject is a human.
- 42. (withdrawn) The method of claim 39 or 40, wherein said subject suffers from or is at risk for a hematopoietic disorder.
- 43. (withdrawn) The method of claim 39 or 40, wherein said subject suffers from or is at risk for a cancer.
- 44. (withdrawn) The method of claim 43, wherein said cancer is a blood cancer
- 45. (withdrawn) The method of claim 39, wherein said method comprises (a) providing said stem cells ex vivo; (b) contacting said cells with said agent under conditions sufficient to increase cell-surface expression of said stem cells; and (c) introducing said contacted cells into said subject.
- 46. (withdrawn) A method of treating a hematopoietic disorder in a subject, the method comprising administering to said subject an agent that decreases the cell-surface or expression of the glycosylated polypeptide of claim 1 in said subject.
- 47. (withdrawn) A method of treating a hematopoietic disorder in a subject, the method comprising: (a) providing blood from said subject (b) contacting said blood with one or more agents that specifically bind to the glycosylated polypeptide of claim 1 under conditions sufficient to form a complex between said one or more agents and a blood cell, if present, in said population of cells; (c) detecting said complex; (d) removing said complex from said blood, thereby removing said blood cell from said blood (e) re-introducing said blood into said subject

Attorney's Docket No.: 10286-014001 / BWH-729

Applicant: Robert Sackstein Serial No.: 10/042,421 Filed: October 18, 2001

Page : 9 of 18

thereby treating said hematopoietic disorder in said subject.

48. (withdrawn) A method of treating a hematopoietic disorder in a subject, the method comprising: (a) providing blood from said subject and a E-selectin polypeptide immobilized on a solid phase; (b) contacting the solid phase with a said blood wherein the relative movement between the solid phase and the blood is such that shear stress is achieved at the surface of the solid phase; and (c) re-introducing said blood into said subject thereby treating leukemia in said subject.

- 49. (withdrawn) A method of treating a hematopoietic disorder in a subject, the method comprising: (a) providing blood from said subject and a L-selectin polypeptide immobilized on a solid phase; (b) contacting the solid phase with a said blood wherein the relative movement between the solid phase and the blood is such that shear stress is achieved at the surface of the solid phase; and (c) re-introducing said blood into said subject thereby treating said hematopoietic disorder in said subject.
- 50. (withdrawn) The method of claim 48 or 49, wherein said shear stress is greater than 0.6 dynes/cm².
- 51. (withdrawn) The method of claim 48, wherein said shear stress is at least 2.8 dynes/cm².
- 52. (withdrawn) The method of claim 49, wherein said shear stress is at least 10.0 dynes/cm².
- 53. (withdrawn) A method of treating an inflammatory disorder in a subject, said method comprising administering to a subject the glycosylated polypeptide of claim 1 or fragment thereof.
- 54. (withdrawn) A method of treating a disorder amenable for treatment with a stem cell in a

Applicant: Robert Sackstein Serial No.: 10/042,421 Filed: October 18, 2001

Page : 10 of 18

subject, the method comprising administering to said mammal a composition comprising the cells isolated according to the method of claim 19, 20, 21 or 22.

55. (withdrawn) The method of claim 54, wherein said disorder is selected from the group comprising myocardial infarction, Parkinson's disease, diabetes, congenital muscle dystrophies, stroke, genetic/congenital disordes and liver disorders.

56. (withdrawn) A method of diagnosing or determining the susceptibility to a hematologic disorder in a subject, the method comprising: (a) contacting a subject derived cell population with one or more agents that specifically bind to the glycosylated polypeptide of claim 1 under conditions sufficient to form a complex between said agent and cell, if present, in said population; and (b) detecting said complex, wherein the presence of said complex indicates the presence of or the susceptibility to a hematalogic disorder in said subject

57. (withdrawn) A method of determining the prognosis or efficacy of treatment of a hematologic disorder in a subject, the method comprising: (a) contacting a subject derived cell population with one or more agents that specifically bind to the glycosylated polypeptide of claim 1 under conditions sufficient to form a complex between said agent and cell, if present, in said population; and (b) detecting said complex, thereby determining the prognosis or efficacy of treatment of a hematologic disorder hematologic disorder in said subject

- 58. (withdrawn) A method of treating a hematopoietic disorder in a subject, the method comprising administering to said subject an agent that specifically bind to the glycosylated polypeptide of claim 1.
- 59. (withdrawn) A method of treating a hematopoietic disorder in a subject, the method comprising administering to said subject an agent comprising a first domain and a second domain linked by a covalent bond, the first domain comprising a compound that specifically bind

Serial No.: 10/042,421 Filed : October 18, 2001 Page : 11 of 18

to the glycosylated polypeptide of claim 1 and the second domain comprising a toxin.

60. (withdrawn) The method of claim 59, wherein the first domain is a HCELL antibody or fragment thereof.

- 61. (withdrawn) The method of claim 59, wherein the toxin is selected from the group comprising Diphtheria toxin (DT) Pseudomonas exotoxin (PE), ricin A (RTA), gelonin, pokeweed antiviral protein, and dodecandron.
- 62. (previously presented) The preparation of claim 1, wherein the polypeptide is a CD44H isoform.
- 63. (previously presented) The preparation of claim 1, wherein the polypeptide is a CD44R2 isoform.
- 64. (new) The preparation of claim 1, wherein the polypeptide is a CD44R1 isoform.